



Overview of Amphibian Workshop

International Workshop on the Use of Anuran Models for
Endocrine Disruption and Reproductive Toxicology
June 2003, Duluth, MN

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International Workshop on the Use of Anuran Models for Endocrine Disruption and Reproductive Toxicology

- Experts from 11 countries participated
- Day 1 - presentations
 - general endocrinology
 - reproductive biology
 - test methods for screening and testing
- Day 2 - breakout group discussions
 - screening assays and testing methods for thyroid axis disruption
 - diagnostic indicators of amphibian endocrine disruption
 - reproductive endocrinology and testing
- Workshop proceedings to be submitted for publication
Fall 2003

Workshop Summary

- Discussion that screening assay is intended as general vertebrate model for effects to thyroid hormone axis
- Choice of test species? *X. laevis* immediately practical, *X. tropicalis* desirable when more information available
- Many endpoints and important MOAs to consider were discussed
- Mechanistic/diagnostic endpoints desirable, but should be balanced with integrative (apical) measures that are simple, unambiguous

Workshop Summary (cont.)

- Proposal to start with NF 51 to continue up to NF60, duration minimum 14 days
- Important considerations and issues were listed (i.e., food/feeding, water quality, mixed MOAs, etc.)
- Flow-through design preferred, but semi-static ok
- Some chemicals identified for key MOAs

Workshop Summary (cont.)

- Tier 2 not focused on general vertebrate screening but for assessing ecological risk in amphibians
- Emphasis for Tier 2 on *Xenopus sp.*, extrapolation critical
- Apical endpoints – fecundity, fertility, hatchability, sexual development & differentiation, and viable offspring
- Full life-cycle or multi-generation desirable, but partial life-cycle more practical

OECD Amphibian Expert Group

The main objectives of the meeting were to:

- discuss and agree on the preferred approach for a frog metamorphosis assay for the detection of thyroid disruptors
- discuss and agree on the main parameters of a protocol
- agree on a timeframe and action plan for further work in the coming months.

Thyroid related modes of action and possible endpoints.

MOA Possible endpoints	Synthesis - I ⁻ uptake - TPO inhibition	Transport - TTR displacement	Elimination - deiodination - conjugation (UDPGT)	Neuro-endocrine HP axis	TR - agonism - antagonism
Morphology: •Dev. Stage •Hind limb	+ ¹ + ²	? ¹ ? ²	+? ¹	+ ¹	+ ¹
Histology: •Thyroid •Pituitary	++ ¹ + ²	? ¹ ? ²	+? ¹	++ ¹ + ²	+ ¹
Biochemistry : •T3/T4 •Enzymes (D2/D3)	+ ²	+ ²	++ ² +?		
Mol. Biology: •TR β mRNA •TSH β mRNA •CRF •UDPGT	+ ¹ + ¹ +	? ¹ ? ¹ ? ?	+ ¹ ? ¹ +	+ ¹ + ¹ 	+ ¹

- + endpoints able to measure effects on the associated thyroid related mode of action;
¹ core endpoints of the frog metamorphosis assay proposed by the Amphibian Expert Group;
² optional endpoints of the frog metamorphosis assay proposed by the Amphibian Expert Group.

Thyroid pathways and relevant chemicals.

Mode of action	Chemicals which have an action
Synthesis	PTU, Methimazole, Perchlorate
Transport	Research need - not known
Elimination	Research need - not known
Thyroid Receptor	NH ₃ , T ₄ , T ₃ , brominated flame retardants (e.g. tetrachlorobisphenol)
Neuroendocrine	Corticosteriod (research need)
Transformation	IOP

Chemicals and countries which conducted the Amphibian metamorphosis assay.

Chemicals	Germany		Japan		United States	
PTU ✓		+		+		+
ETU		+		+		
Zineb		-				
Amitrole		+				
T4 ✓		+		+		+
T3						+
IOP		+				+
Methimazole						+
Perchlorate						+
Estradiol						-
B-trenbolone						-
TBBA		-				

Shaded cell: country used the chemical;

+ chemical came up positive;

- chemical showed no thyroid related effect;

✓ chemicals preferred for the optimization phase

Endpoints	Measurement technique	Priority level	Research needs
Developmental morphology: •Staging (includes hind limb development) •hindlimb length	NF scale Analo/digital calipers/video frame grab and image analysis software	Core Optional	
Thyroid histology	Qualitative Quantitative	Core Core/Optional	•Define whether quantitative evaluation is possible /needed; •Define histological endpoints to be looked at (eg. epithelial cell height)
Pituitary histology		Optional	
Biochemistry: •T3/T4	RIA using whole body/brain	Optional	•Develop a protocol for extraction from whole body homogenates or specific tissues, •Develop a standardized RIA protocol for T3/T4
•Deiodinase activity (D2/D3)	No assay/method available	----- --	
Molecular biology: •TR α -mRNA •TR β -mRNA •TSH β sub-unit-mRNA	Semi-quant ^{ve} PCR Semi-quant ^{ve} PCR Semi-quant ^{ve} PCR	Optional Core <i>spin-off</i> Core <i>spin-off</i>	Need to establish the expression profiles of the biomarkers throughout metamorphosis

Additional Work

- Protocol optimization underway- complete Dec 2003
 - Compare protocols beginning NF 51 continue for 21 days and beginning NF 54 continue for 14 days
 - US, Germany, Japan participating
- Draft protocol for validation work Jan 2004
- Interlaboratory validation exercise Summer 2004